REMARKS

In the Office Action mailed September June 29, 2004, claims 1-46 were pending, with claims 1-45 rejected therein and claim 46 withdrawn by the Examiner. With entry of this amendment, claims 2, 4-5, 8-9, 18-20, 26-45 and 47-63 are pending. Claims 1,3, 6-7, 10-17, 21-25, and 46 are cancelled without prejudice or disclaimer. Applicants reserve the right to pursue the subject matter of claim 46 in one or more continuation applications. Claims 2, 4, 8, 9, 18-20, 28, 30-36, 38-40, 42, and 45 are amended. Claims 47-63 are added. Applicants submit the new claims are supported throughout the specification including the original claims. Applicants request entry of this Amendment and Response and reconsideration of the rejection of the claims.

The Applicants acknowledge and thank the Examiner for the consideration and return of the Information Disclosure Statements of December 26, 2001, and September 6, 2002. Please note that the first 3 patent references listed on the December 26, 2001 were not initialled. Applicants further note the Sequence Listing filed complies with the requirements and has been entered.

In the Specification

The specification is amended to provide the further description of the drawings as requested by the Examiner. Brief Description of the Drawings for Figures 1a-1e, 3a-3e, 8a-8f, 9a-9d, 10a-10e, 12a-12e, and 13a-13b is amended. In addition, the description for Figures 24, 25a, and 25b, are amended to correct a spelling error.

The descriptor for Figure 7b is amended to include the sequence identifier. This amendment is made in response to the Examiner's objection that some of the drawings have nucleic acid sequences but lacked the appropriate sequence identifiers. MPEP 2422.02 indicates the sequence identifiers may be provided either in the figure itself or in the Brief Description of the Drawings. The descriptors for Figures 11, 21 and 25 were amended in the substitute specification filed on May 24, 2002, to include the appropriate sequence identifiers for these sequences. Applicants respectfully submit they are now in compliance with the sequence listing requirements and respectfully request the removal of this objection.

The Examiner noted that claim 18 included an improper markush format. This objection has been address in the amendments to the claims.

The Abstract has been combined into a single paragraph to comply with the Examiner's requests.

In The Claims

35 U.S.C § 112

Claims 1-45 are rejected under 35 U.S.C § 112, second paragraph, as being indefinite. The rejection was subdivided into letter marked paragraphs. This convention will be continued in the remarks below.

A) Claims 1-45 are rejected as indefinite over the recitation of "the DNA", because it is not clear as to whether "the DNA" refers to the amplified product or the DNA from the sample. In response to this rejection, new claim 47 has been presented to provide clarity on the DNA containing sample, the DNA from it, the amplified product, and the further amplified products so to address the rejection.

B) Claims 1-45 are rejected as indefinite over the recitation of "examining one or more characteristics" because it is not clear as to what is encompassed by "characteristics of the further amplified products". Claim 1 is replaced by new claim 47 which provides further guidance by referring to the use of "the presence or absence of a distinctive unit to examine the characteristics." Applicants assert it is now clear that a distinctive unit is used to examine the characteristics of the SNP, i.e. the nucleotide identity of the SNP.

C) Claims 1-45 are rejected as indefinite over the recitation of "one side of the SNP under investigation" as it is allegedly not clear as to what is encompassed by "one side of the SNP under investigation." The language indicated by the Examiner was found, in claim 1, now cancelled. Response to this rejection is made to the extent that it now applies to language present in new claim 63. The concept of annealing to one side of a SNP is not unclear. An SNP, by definition, is found within a region of DNA. Therefore, on either side of an SNP, are additional nucleotides (bases). The phrase annealing to one side of the single nucleotide polymorphism

under investigation simply refers to annealing to bases adjacent to the SNP on one side or the other on the DNA strand.

D) Claims 1-45 are rejected as indefinite because claim 1 is drawn to a method for investigating SNP, however, the final step is for examining one or more characteristics of the further amplified product. This rejection has been obviated by cancelling claim 1 and adding claim 47.

E) Claims 1-45 are rejected as being indefinite over the recitation of "the SNP" because this recitation lacks antecedent basis. This rejection is obviated by the cancellation of claim 1 and the addition of claim 47. Furthermore, claim 2 has been amended to provide antecedence.

F) Claim 2 is rejected as being indefinite over the recitation of "the primers" because this recitation lacks antecedent basis. Claim 2 has been amended to depend from claim 47, thereby providing the necessary antecedence.

G) & H) Claim 2 is rejected as being indefinite over the recitation "provided with an SNP portion" and "SNP identity portion" for being unclear. Furthermore, "the primer with an SNP identity portion" is rejected for a lack of antecedent basis. Claim 2 has been amended to recite an SNP identifying portion in each case. Support for the SNP identifying portion is provided in the specification, for example in particular, at page 3, lines 3 and onward. The SNP identifying portion is a nucleotide that pairs to the SNP, e.g., C to G. This function is further explained in the context of Figures 3a - 3e. The amendment to claim 2 also obviates the antecedence issue.

I) The rejection of claim 3 has been obviated by its cancellation.

J) Claim 4 is rejected as indefinite over the recitation "which matches the sequence of the locus sequence in the vicinity of the SNP under investigation..." because it is not clear as to what "matching sequences" are, what constitutes the "vicinity of SNP under investigation", and it is not clear as to what " the locus commencing at between 1 and 10 bases to the respective sides of the SNP" is. Although the Applicants assert that the intention behind the term "match" is quite clear, claim 4 is amended to substitute "pairs to" for "match", so as to reflect the annealing which is illustrated in Figure 3a and described in the accompanying specification

description. The "vicinity of the SNP under investigation" indicates where the annealing is expected to occur. This phrase is further modified by the locus commencing at between 1 and 10 bases to the respective sides of the SNP. For example, a single base SNP identifying portion pairs with a base adjacent the SNP. Then SNP identifying portion may be greater in size, thereby, pairing/annealing further away from the SNP, e.g., between 1 and 10 bases to the respective sides of the SNP.

K) Claim 5 is rejected as being indefinite over the recitation of "for each possible identity of the SNP" as being unclear. As is understood from the art, the possibilities for the identity of an SNP are one of the 4 bases found in DNA, i.e., A, G, C, or T.

L) Claims 6 and 7 are rejected as being indefinite over the recitation "the further portion...by SNP related portion" as being unclear. Claims 6 and 7 are cancelled thereby obviating the rejection.

M) & O) The rejections of claims 7 and 12 are obviated by cancellation of claims 7 and 12.

N) Is omitted in the Office Action.

P) Claim 8 is rejected as being indefinite over the recitation of "a set" because it is not clear as to whether it refers to the first set of primers or some other set of primers. Claim 8 has been amended to clarify that it is referring to the first set of primers.

Q) Claim 8 is rejected as being indefinite over the recitation of "provided with identical sequences of each primer" because it is not clear as to whether the primers in the set are identical or the region containing the locus specific portion is identical for all primers. Claim 8 is amended to make clear that the primers for a particular SNP have the same locus specific sequence, but will differ in terms of the SNP identifying portion.

R) Claims 9 and 10 are rejected as being indefinite over the recitation of "match" and "sequence matching" as being unclear. Claim 9 is amended to replace the term "match" with the term "pairs". Claim 10 has been cancelled.

S) Claims 9 and 10 are rejected as being indefinite over the recitation of "the locus sequence" for lack antecedent basis. Claim 9 has been amended to provide antecedence and claim 10 is cancelled.

T) thru Z) Rejections T thru Z were directed to claims 10 -16. These rejections are obviated by cancellation of the claims.

AA) Claims 17-20, 38-40, and 45 are rejected as being indefinite over the recitation of "a distinctive unit" as being unclear. The phrase "a distinctive unit" is introduced, discussed, and exemplified within the application as filed. In particular, see page 13 and 14 of the specification. "A distinctive unit" is a reasonable generalization of the requirement for this feature. It provides an indication which is detectable and distinct for identification of one SNP as compared with a different one.

AB) Claims 17-20 are rejected as being indefinite because of language in 17: "one or more characteristics of the further amplified products are investigated by means of the presence and/or absence of the distinctive unit." The rejection of claim 17 is obviated by the cancellation of the claim. Claims 18-20 are amended to be dependent from claim 47, which provides further clarification regarding examination of one or more characteristics through use of a distinctive unit, thereby obviating this rejection. Withdrawal of this rejection is respectfully required.

AC) Claim 18 is rejected as being indefinite over the recitation of "characteristic isotope" as being unclear. Claim 18 is amended to remove the objected to term, as the subject matter thereof is encompassed by the emitter of radiation.

AD) Claim 19 is rejected as being indefinite over the recitation of "second set" for lack of antecedent basis. Claim 19 is amended to provide the required antecedence.

AE) Claim 20 is rejected as being indefinite over the recitation of "the distinctive unit is indicative if the nucleotide presence of the SNP" because it is not clear how the presence and/or absence of the "distinctive unit" is "indicative of the nucleotide presence of the SNP." Furthermore, it is not clear to the Examiner what "the nucleotide presence" of the SNP is. The manner in which a distinctive unit indicates a particular SNP is present is clear from the specification. The particular distinctive unit incorporated into the final product is dependent upon the identity of the SNP being investigated. Thus, a different distinctive unit is incorporated

where the SNP is C, compared with a DNA sample where the SNP is G. This function is described, for example, in the embodiment presented in pages 28-29 and is further illustrated in Figure 3. Withdrawal of this rejected is respectfully requested.

AF) Claims 21, 25, and 42 are rejected as being indefinite over the recitation of "of at least in part" as being unclear. The rejection of claims 21 and 25 is obviated by cancellation of the claims. Claim 42 has been amended by reordering the language to make clear that the probes are at least slightly different from each other in their sequence.

AG) Rejections AG, AH, AI, AJ, and AK are directed to claims 22-25. These rejections are obviated by the cancellation of claims 22-25.

AL) Claims 28-29 are rejected as being indefinite over the recitation of "at least some of the cycles of the amplification process is such that at least 80% of the second set of primers remain single stranded" as being unclear as to how many cycles are referred to and how one determines when at least 80% of the second set of primers remain single stranded. Claim 28 has been amended to make clear that the requirement applies to one or more of the cycles. The extent of the single strandedness of the second set of primers can be determined in a number of ways, for example, by evaluation the extent of the annealing at different temperatures or alternatively, by experimentally examining the progress of the amplification by performing a number of experiments and evaluating the number of copies produced. The number of copies produced after a no-number of cycles is a reflection of the extent of the annealing in those cycles and this is directly related to the extent of single strandedness. These two terminations are apparent in the art. Withdrawal of this rejection is respectfully requested.

AM) Claim 30 is rejected as being indefinite as claim 1 does not refer to "cycles" and it is "annealing temperature" is related to claim 1. Claim 30 has been amended to provide antecedence for the various terms and to redirect dependency to claim 47.

AN) Claims 31-32 are rejected as being indefinite over the recitation of "the annealing temperature" and the "amplification process" for lack of antecedent basis. Claims 31 and 32 are amended to provide antecedence.

AO) Claim 33 is rejected as being indefinite for being unclear as to whether the amplification products in the second set of primers are separated or one or more second sets of primers are separated. Claim 33 has been amended to make clear that the amplification products are separated from one another.

AP) Claims 34-36 are rejected as being indefinite over the recitation of "having a sequence which anneals with at least part of the sequence of one of the further amplified products" because it is not clear as to what "at least part of the sequence" refers to. Applicants respectfully disagree that these concepts are unclear. "Having a sequence which anneals with at least part of the sequence of one of the further amplified products" requires for sufficient links of the component sequence and further amplified product to hybridize thereby retaining the further amplified product on the component, so that it is not washed away during subsequent steps. This may be achieved through a hybridization of a part or the whole of the sequences, as is generally understood. The term "retained on a solid support" means that the components are fixed to such an extent that they remain on the solid support when other elements are free to leave, for instance, during the washing away of uncaptured probes, etc.; see Figures 10e and 12d. The capture of biomolecules through binding or hybridization processes to a solid support for purification is a generally understood concept.

AQ) Claim 35 is rejected as being indefinite over the recitation of "the bases before the base which is the SNP side" as being unclear. Claim 35 has been amended to remove the typographic error which was the inclusion of "side" after SNP. With this correction of claim 35, it is believed that the references relative to the SNP location are now clear.

AR) Claim 36 is rejected as being indefinite over the recitation of "anneals to the further amplified product along the sequence corresponding..." as being unclear. Claim 36 has been amended to clarify the parts of the further amplified product that are hybridized to the contained component.

AS) Claim 38 is rejected as being indefinite over the recitation of "further components to introduce a distinctive unit" as being unclear. Components are described within the specification, for example, at page 15 and 16. Further components may include dideoxynucleotides and oligonucleotides, and potentially may include dyes or other forms of

distinctive units. Ligation, hybridization and other methods to introduce the distinctive unit to the component are described in detail in the specification. Distinctive unit introduced, discussed and exemplified within the specification as filed, in particular, see pages 13 and 14.

AT) Claim 40 is rejected as being indefinite over the recitation of the "end base" as lacking antecedent basis. Claim 40 has been amended to provide proper antecedence.

AU) Claim 45 is rejected as being indefinite over the recitation of "a distinctive unit relative to each other" as being unclear. Claim 45 has been amended to clarify its meaning.

Withdrawal of the rejections made under §112 ¶2 are respectfully requested in view of the comments above.

35 U.S.C § 102

Claim 1-16 and 21-45 are rejected under 35 U.S.C § 102(b) as being anticipated by Paranavitana (Molecular and Cellular Probes (1998) 12:309-315). Applicants respectfully traverse this rejection.

The rejection asserts Paranavitana teaches a method of detecting point mutations in a DNA sample by amplification with a first set of primers to give a first product; contacting the first product with a second set of primers to give a further amplified product and examining the presence or absence of K-ras point mutations using dot blot hybridization wherein the PCR products are attached to a solid support and 3' labelled with Digoxigenin-11 ddUTP oligonucleotides probes. The rejection also asserts that first set of primers allegedly comprise a "locus specific portion" (allele specific primer, Primer A) and a "further portion" (codon 12 second position common primer B). Applicants respectfully disagree.

Under 35 U.S.C. §102, "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

The present invention is directed to a method that may be used for investigating one or more SNPs in a DNA sample by the inclusion primers for each SNP to be investigated in the first

set of primers, and then amplifying the first product with the second set of primers, and detecting each SNP by hybridizing to a component with a distinctive unit. By utilizing different combinations of distinctive units attached to components for hybridization with different further portions, the identity of each SNP is indicated.

The claims are directed to a method of investigating SNPs with a first set of primers to give a first amplified product, wherein at least one primer has a locus specific portion and a further portion, and wherein the first amplified product includes a sequence complementary to the locus specific portion and the further portion of a first set primer. The further portion does not anneal with the locus. The first amplified product is amplified by annealing a second set of primers to the sequence complementary to the further portion to give a further amplified product.

The further amplified product is examined for one or more characteristics indicated by the presence or absence of a distinctive unit introduced by hybridisation or annealing of a component attached to a distinctive unit to the sequence complimentary to the further portion.

In contrast, *Paranavitana* teaches a two –step PCR procedure for detection of K-ras mutation in human colon tumor samples. The first PCR amplification is of exon 1 of the K-ras gene. A subsequent amplification of the product uses allele specific K-ras primers to detect mutations at codon 12. The mutations were confirmed by non-radioactive oligonucleotide hybridization with allele-specific probes. (Page 313, col. 2- page 314.)

In *Paranavitana,* the first amplification uses only two primers, the forward primer of page 310, right hand column, line 14 and reverse primer of page 310, right hand column, line 25, to amplify the gene containing codon 12. A number of samples of this amplified product are taken and contacted with a second set of primers. The second set of primers consists of primer A and one of primer A1, A2, A3 or A4. For a full test samples are processed using four combinations, A and A1, A and A2, A and A3, A and A4. In each case primer A provides the forward primer whilst one of A1, A2, A3 or A4 provide the allele specific reverse primer.

The further amplified product for each of the samples are then subjected to electrophoresis in their own channel of a gel, see Figure 1, to visualise the amplification products present. Thus in the case of channel 2 of Figure 1, the wild type glycine is indicated as present in

the sample; in the case of channel 3, valine is indicated as absent; in the case of channel 4, alanine is indicated as absent; and in the case of channel 5, aspartate is indicated as present. Each channel needs to represent the result of amplification using only A and one of A1, A2 etc as otherwise there would be no way of knowing which primer A1, A2 etc had generated the observed band.

With respect to the second position investigated on codon 12, a series of separate samples are taken and contacted with B and B1, B and B2, B and B3, B and B4 in the same way. In this case, primer B provides the reverse primer, one of primers B1, B2, B3 or B4 provide the allele specific forward primer. The separate nature of the contact with primer set A and primer set B is also confirmed by the different conditions for the amplification which are provided depending on whether second set A or second set B is being used; bottom of page 310, right hand column.

The Paranavitana reference does not teach the claimed requirement for *a distinctive unit introduced by hybridisation or annealing of a component to the sequence complimentary to the further portion*. P*aranavitana* does not describe a distinctive units for the identification of SNPs. Instead, it relies upon a physical separation of the samples and separating the different primers into separated samples. This means that the test must be performed as a singleplex. Multiple SNP's cannot be investigated in the same sample at the same time, because there is no way of knowing whether the band arose from one SNP target or another.

Paranavitana does not teach or disclose hybridisation or annealing to the sequence complimentary to the further portion. In *Paranavitana* both the primers of the first set are intended to fully anneal to the DNA sequence including the target. As such no further portion is provided as a part of the first set primers. This then means that there is no sequence complimentary to it in the further amplified product.

In contrast, the claimed method provides the ability to investigate multiple SNPs in a sample by using two or more first primers each incorporating a further portion and through use of distinctive units. The present invention ensures that the second set of primers need only provide two forward primers to amplify the amplified product, even where that amplified product contains a number of different sequences, each corresponding to a different target. This is because all the different sequences include one of the two common further portions. The second

stage thus provides amplification with a small number of primers and ensures even amplification efficiency for each of the targets, and without the need to balance a large number of second set primer performances relative to one another.

The above-mentioned differences between the claimed invention and *Paranavitana* are supplemented by the following differences:

Paranavitana teaches two primers for use in the first amplification (primers A & B). The two primers in the first set are formed of a locus specific portion only as the entire primer A and entire primer B are intended to anneal during PCR. Paranavitana does not teach inclusion of additional features such as is required in the claims. The claims require "two or more primers including a locus specific portion and a further portion, wherein, *the further portion of at least one of the primers being different from the further portion of at least one of the other primers*". Paranavitana does not teach a further portion and does not provide for further portions being different between the primers.

Paranavitana does not teach each and every element of the claims, and therefore does not anticipate claims 1-16 and 21-45. Removal of the rejection is respectfully requested.

35 U.S.C § 103

Claims 17-20 are rejected under 35 USC §103a as being unpatentable over Paravitana, as applied to claims 1-16 and 21-45 above, and in view of Wu et al. (PNAS (1989) 86:2757-2760). Applicants respectfully traverse.

The rejection asserts Paranavitana teaches a method of detecting point mutations in a DNA sample by amplification with a first set of primers to give a first product; contacting the first product with a second set of primers to give a further amplified product and examining the presence or absence of K-ras point mutations using dot blot hybridization wherein the PCR products are attached to a solid support and 3' labelled with Digoxigenin-11 ddUTP oligonucleotides probes. The rejection also asserts that first set of primers allegedly comprise a "locus specific portion" (allele specific primer, Primer A) and a "further portion" (codon 12 second position common primer B). Applicants respectfully disagree.

The rejection further indicates that Paranavitana does not teach the investigation of a SNP by means of the presence or absence of a distinctive unit in the further amplified product. The rejection asserts Wu teaches performing allele specific PCR using 2 primers, one for the normal allele and one for the sickle cell allele (polymorphism) by differentially labelling the PCR primers and thereby labelling the PCR product.

In order to establish a prima facie case of obviousness, three basic criteria must be met, namely: 1) the references when combined must teach or suggest all of the claim limitations; 2) a suggestion or motivation to modify the references or combine the reference teachings must be present; and 3) the references when combined must provide a reasonable expectation of success. Applicants submit that all of these requirements have not been met.

As described above, and admitted by the Examiner, Paranavitana does not teach or suggest the use of a distinctive feature to identify SNPs. Furthermore, Paranavitana does not teach or suggest primers incorporating further portions for hybridization with a component including a distinctive feature. Combining Paranvitana with Wu does not correct these deficiencies. Wu does not teach or suggest primers incorporating further portions for hybridization with a component including a distinctive feature.

The combination of *Paranavitana* with *Wu* is not a technically feasible combination and furthermore, there is no motivation to combine Paranavitana with Wu. Firstly, *Paranavitana* detects the target of interest by monitoring for non-amplification as much as for amplification. A distinctive unit is of no assistance in such a system. Secondly, *Paranavitana* uses two amplification stages whereas *Wu* uses only one. There is no clear pointer as to which stage the distinctive unit would be used in, therefore, and no teaching as to how.

Most importantly of all, the claimed invention requires that the distinctive unit be introduced using *the sequence complimentary to the further portion*. As stated above, this is not taught or suggested in *Paranavitana* as there is no such further portion there. Furthermore, *Wu* does not assist in any way, as in *Wu* the teaching is that the distinctive unit is part of the first primer and so can only be introduced by annealing to the actual DNA sample. *Wu* does not address the missing concept of the use of the further portion therefore. As discussed above, it is

the use of this mode for introducing the distinctive feature which gives the technique its advantages in being useful as a multiplex and in consistent amplification and detection of SNPs.

The combination of Paranavitana with Wu fails to teach all the elements of the claims and therefore does not render the claimed invention obvious. Reconsideration of the claims in view of the arguments presented above and removal of the rejection is respectfully requested.

If a telephone conference would be helpful in resolving any issues concerning this communication, please contact Applicants' below-signed attorney.

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